Journal of Chromatography, 368 (1986) 423–426
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 964

Note

Application of column switching in high-performance liquid chromatography to arsenic speciation analysis with inductively coupled argon plasma spectrometric detection

GARY K.-C. LOW*, GRAEME E. BATLEY and STEPHEN J. BUCHANAN
CSIRO Division of Energy Chemistry, Private Mail Bag 7, Sutherland, N.S.W. 2232 (Australia)
(Received July 21st, 1986)

The resolution of co-eluting or partially resolved chromatographic peaks can often be enhanced by using two or more columns with selective switching of the mobile phase from the outlet of one column to the inlet of successive columns having different adsorptive properties. Although widely accepted in gas chromatography[1,2], column switching is less frequently used in high-performance liquid chromatography (HPLC) because of the stringent requirements imposed when different modes of HPLC columns are involved. Solvent compatibility, especially solvent miscibility, and rapid column conditioning are of critical importance for successful application of this technique[3,4].

The separation of arsenic species in biological samples using HPLC has been the subject of numerous studies[5–7], although few systems have been able to resolve successfully all commonly encountered species: arsenic(III), arsenic(V), monomethylarsonic acid, dimethylarsinic acid and arsenobetaine. In this study column switching between a reversed-phase C_{18} column and an anion-exchange column is explored to resolve these species. An inductively coupled plasma atomic emission spectrometer (ICPAES) has been used as the detection system rather than the conventional hydride generation atomic absorption detection as arsenobetaine does not form a hydride and is not detected by the latter technique.

EXPERIMENTAL

Reagents and standard solutions

Stock standard solutions (As concentration: 750 mg l^{-1}) were prepared from sodium arsenate (Merck, Darmstadt, F.R.G.), sodium arsenite (BDH, Poole, U.K.), sodium methylarsonate (Alfa Ventron, Danvers, MA, U.S.A.), sodium dimethylarsinate (Fluka, Buchs, Switzerland) and arsenobetaine (donated by Dr. R. Toia, University of New South Wales, Australia) in prefiltered and distilled water. Appropriate dilutions were made immediately before use. The eluting mobile phase for HPLC was 3 m*M* ammonium dihydrogen orthophosphate adjusted to pH 6.0 by either concentrated ammonia or orthophosphoric acid.

Unless otherwise stated, all chemicals were analytical reagent grade.

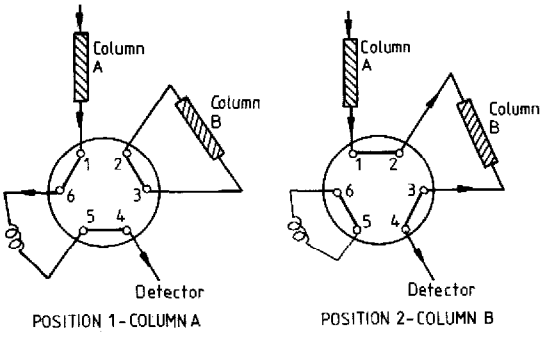
0021-9673/86/$03.50

Instrumentation

The HPLC system consisted of a Waters Model M45 solvent delivery system, a Waters UK6 injector, a 25 cm × 4.1 mm I.D. Hamilton PRP-X100 anion chromatography column (Hamilton, Reno, NV, U.S.A.), a 25 cm × 4.1 mm I.D. Vydac 201TP 5 μm reversed-phase column, and a Waters Guard-Pak Precolumn Module with C_{18} insert cartidges. The columns were connected to a Waters 6-port automated switching valve and the arrangement of the columns and the positions of the switching are shown in Fig. 1. The outlet from this valve was connected to the nebulizer system of an ICPAES[8] with PTFE tubing (25 mm × 0.2 mm I.D.). Since all of the eluent flowed into the nebulizer, its uptake rate was the same as the eluent flow-rate, set at 2 ml min^{-1}. Arsenic was monitored at 228.8 nm. The optimized ICP parameters were 0.9 kW radio frequency (rf) power, samples gas flow-rate 1 l min^{-1}, and observation height 14–18 mm. The detection signal was recorded simultaneously by a Watanabe Servorecorder SR6312 and a locally built STD bus-based microcomputer which was used to quantify the area of the recorded peaks.

RESULTS AND DISCUSSION

Experiments using an anion-exchange HPLC column with a range of solvents for the separation of the five arsenic compounds failed to resolve successfully all species, with arsenobetaine typically co-eluting with arsenic(III) (Fig. 2A). A reversed-phase C_{18} column under certain solvent conditions was able to separate arsenobetaine, but could resolve the remaining four species into only two peaks (Fig. 2B). It was conceivable therefore that switching of the co-eluting peaks from the



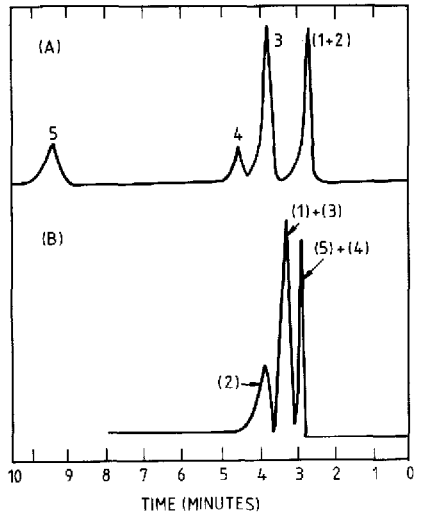


Fig. 1. Switching valve configurations used for diverting flow from the anion-exchange column (A) to the reversed-phase column (B).

Fig. 2. Separation of arsenic species on the anion-exchange (A) and reversed-phase (B) columns, using 3.0 m*M* ammonium dihydrogen phosphate pH 6, at 2 ml min^{-1}. 1 = As(III); 2 = arsenobetaine; 3 = dimethylarsinic acid; 4 = monomethylarsonic acid; 5 = As(V).

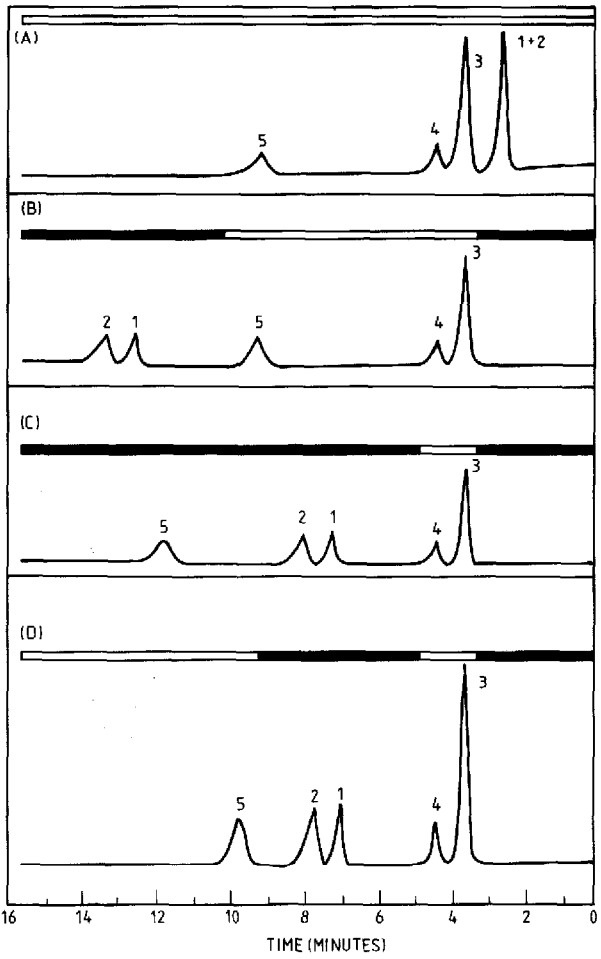


Fig. 3. Separation of arsenic species using selected column switching sequences. Switch position: □ position 1; ■ position 2 (Fig. 1). 1 = As(III); 2 = arsenobetaine; 3 = dimethylarsinic acid; 4 = monomethylarsonic acid; 5 = As(V).

anion-exchange column to the reversed-phase column might facilitate their resolution.

To achieve this, the chosen mobile phase must be compatible with both columns. For anion-exchange separation of arsenic species, a low ionic strength electrolyte buffered between pH 6 and 8 achieves the best separation[7]. The constraints on the reversed-phase column are more critical, with respect to both pH and electrolyte composition. An ammonia buffer was selected for this study. Its advantages in reversed-phase HPLC has been reported previously[9], while it is also fully compatible with the anion-exchange column.

Modes of column switching

The use of single mobile phase for a column-switching system permits the

switching to different modes of separation at any time without the need for column reconditioning. Careful planning of the column switching should effectively reduce the analysis time. Fig. 3 shows the results of the separation of the standard mix using different switching sequences.

With the switching valve initially set at position 2 (Fig. 3B), the unresolved As(III) and arsenobetaine peak could be diverted to the reversed-phase column. The switching valve was then turned to position 1 for the separation of monomethylarsonic acid, dimethylarsinic acid and As(V) on the anion-exchange column, then returned to position 2 to allow the final separation of As(III) and arsenobetaine. The analysis time can be reduced further by diverting the elution of As(III) and arsenobetaine in the elution interval between dimethylarsinic acid and As(V). This can be achieved in three ways:

(a) The separation of dimethylarsinic acid, monomethylarsonic acid and As(V) is allowed to occur in the anion-exchange column and the remainder are eluted from the combined columns (Fig. 3B).

(b) Dimethylarsinic acid and monoethylarsonic acid only are eluted from the anion-exchange column and the other species from the combined column (Fig. 3C).

(c) As in (b) but switching to the anion-exchange column alone after elution of the arsenobetaine (Fig. 3D). This configuration is only possible when the retention time of As(V) on the anion-exchange columns alone exceeds the elution time of arsenobetaine in the combined columns.

Optimization of the switching technique in this manner can significantly reduce the analysis time resulting in sharper peaks and a decreased detection limit. The technique has been applied to urine samples after preliminary preconcentration. Calibration plots of peak areas versus arsenic injected were linear and coincident for each arsenic species in a spiked urine sample.

Although the switching of the columns was carried out manually in this work, it can be completely automated using, for example, the Waters 840 Data and Chromatography Control Station[10] which provides facilities for the time-switching operations to be written into the control software.

REFERENCES

1 D. W. Wright, K. O. Mahler and L. B. Ballard, *J. Chromatogr. Sci.*, 24 (1986) 60–65.
2 F. Muller and U. K. Goekeler, *Int. Lab.*, June (1985) 42–55.
3 J.-B. Lecaillon, N. Febvre and C. Souppart, *J. Chromatogr.*, 317 (1984) 493–506.
4 G. S. Clarke and M. L. Robinson, *Anal. Proc.*, 22 (1985) 137–138.
5 K. A. Francesconi, P. Micks, K. A. Stockton and K. J. Irgolic, *Chemosphere*, 14 (1985) 1443–1453.
6 R. A. Stockton and K. J. Irgolic, *Intern. J. Environ. Anal. Chem.*, 6 (1979) 313–319.
7 C. T. Tye, S. J. Haswell, P. O'Neill and K. C. C. Bancroft, *Anal. Chim. Acta*, 169 (1985) 389.
8 L. S. Dale and S. J. Buchanan, *J. Anal. Atom. Spectrom.*, 1 (1986) 59–62.
9 C. K. Lim and T. J. Peters, *J. Chromatogr.*, 316 (1984) 397–406.
10 *Waters V840 Data and Chromatography Control Station Operation and Reference Manual*, Manual Number 87552, Waters Associates Publications Department, Milford, MA, revision A, October, 1984 (3/84).